

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) Publication number:

0 351 891 B1

(12)

EUROPEAN PATENT SPECIFICATION(43) Date of publication of patent specification: **29.09.93** (51) Int. Cl.⁵ **G01N 27/30, C12M 1/40**(21) Application number: **89116797.5**(22) Date of filing: **08.05.84**(30) Publication number of the earlier application in accordance with Art.76 EPC: **0 127 958**(54) **Printed electrodes.**

(30) Priority: **05.05.83 GB 8312262**
05.05.83 GB 8312261
06.09.83 GB 8323799
16.12.83 GB 8333644
11.01.84 GB 8400650
29.02.84 GB 8405262
29.02.84 GB 8405263

(43) Date of publication of application:
24.01.90 Bulletin 90/04(45) Publication of the grant of the patent:
29.09.93 Bulletin 93/39(64) Designated Contracting States:
BE CH DE FR GB IT LI NL SE

(56) References cited:
EP-A- 0 030 503 EP-A- 0 078 636
EP-A- 0 152 541 DE-A- 2 127 142
US-A- 4 185 131 US-A- 4 224 125
US-A- 4 229 490 US-A- 4 376 689

(73) Proprietor: **MediSense, Inc.**
266 Second Avenue
Waltham Massachusetts 02154(US)

(72) Inventor: **Hill, Hugh Allen Oliver**

Nine Clover Close
Oxford OX2 9JH(GB)
Inventor: **Higgins, Irving John**
Cotswold Graze Hill
Ravensden Bedford MK44 2TF(GB)
Inventor: **McCann, James Michael**
33 Dewhurst Road
West Kensington London W14 0ES(GB)
Inventor: **Davis, Graham**
1504 Fox Run Drive
Plainsboro NJ 08536(US)
Inventor: **Treidl, Bernhard Ludwig**
15528 Grinnel Terrace
Derwood Station MD 20855(US)
Inventor: **Birket, Nigel Norman**
Drayton House Station Road
Bloxham Banbury Oxon(GB)
Inventor: **Plotkin, Elliot Verne**
14 George Street
Bedford MK4 3SG(GB)
Inventor: **Zwanziger, Ron**
322 Waverley Avenue
Newton, MA 02158(US)

(74) Representative: **Ruffles, Graham Keith et al**
MARKS & CLERK 57-60 Lincoln's Inn Fields
London WC2A 3LS (GB)

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

European Patent specification EP-152541-A published 28 August 1985 describes the manufacture of an amperometric electrode based on enzyme catalysis of a reaction of a substrate present in solution. Working and reference electrodes are formed as thin deposits on an inert base of sintered ceramic oxide by screen printing, using inks respectively comprising on the one hand a platinum powder and a glass powder base, and on the other hand a silver powder and a glass powder base. The deposits are then fired at elevated temperature. The sintered ceramic oxide base is porous or a glass or porous vitroc ceramic membrane is formed on the working electrode. An activated silane is grafted onto the porous base or the porous membrane, and at least one enzyme is then grafted onto the silane. Grafting of the enzyme is typically achieved by immersion of the porous silanized membrane in a solution of the enzyme for 24 hours at 4 °C.

German Offenlegungsschrift 2127142 describes the fabrication of analytical devices comprising an electrically insulating base member, a pair of spaced electrically conductive thin film electrode elements carried on the base, and an overlying semi-permeable matrix containing a test reagent. Typically, metal film is deposited by thermal evaporation onto a ceramic base and then etched using photoresist printing techniques to form the spaced electrode elements. The matrix with enzyme as test reagent is then applied by dip coating.

US Patent 4224125 describes an enzyme electrode in which an oxidoreductase enzyme and a redox compound acting as mediator are immobilized on or in the neighbourhood of an electrode. Various methods are described for immobilization of the enzyme and/or mediator, including covalent bonding of the mediator to the electrode, immobilization of the enzyme by cross-linking, and physical entrapment of the mediator and enzyme as a gel in a defined space adjacent the electrode. In this last instance, the mediator is in the form of a redox polymer, rendering it insoluble, and Example 2 discloses the inclusion of carbon powder in the gel.

In accordance with the present invention, there is provided a suspension formed as a printable and conductive ink for use in the fabrication of electrodes, the suspension comprising a liquid medium containing (a) carbon, together with at least one of (b) an enzyme and (c) a mediator compound.

Both an enzyme and a mediator can be present in the ink.

In a preferred aspect, the enzyme is glucose oxidase and the mediator is ferrocene or a ferrocene derivative.

The present invention further provides for the use by printing of the suspensions of this invention, in the fabrication of an electrode.

The present invention also provides a method of manufacturing a sensor electrode system which is generally elongate and thereby manipulable, the sensor electrode system comprising an electrode carrier in the form of a flat strip carrying a working electrode and a reference electrode in spaced non-contiguous relationship, in which method an enzyme and/or a mediator are applied to the flat strip by printing a suspension of the present invention.

The suspensions provided by the present invention can be used for example in the manufacture of electrode sensor systems for selective detection, measurement or monitoring of a given dissolved substrate in a mixture of dissolved substrates, comprising:-

(a) an area of first electrode material comprising an enzyme catalytic of the said substrate and a mediator compound to transfer charge to the electrode when the enzyme is catalytically active, adjacent to but non-contiguous with;

(b) an area of reference electrode material; both electrodes being of small dimension, and extending as or supported on an elongate member to facilitate manipulation before or during contact with live tissue or a small withdrawn sample of body fluid.

The sensor electrodes can be dipped into or similarly contacted with a liquid substrate e.g. a glucose-containing small blood sample or drop of blood.

In one aspect, a sensor can be manufactured for contact with a liquid mixture of components for detecting the presence of, measuring the amount of and/or monitoring the level of one or more selected components capable of undergoing an enzyme catalysed reaction, the sensor comprising:-

(a) an elongate support member,

(b) on a surface thereof towards one end an expanse of a first electrode of electrically conductive material comprising at least at an outer surface thereof the combination of an enzyme and a mediator compound which transfers electrons to the first electrode when the enzyme is catalytically active,

(c) on a surface of the elongate support member and also towards the said end thereof to be in close proximity therewith an expanse of a second, reference, electrode, and

(d) separate electrical connection to each electrode for attachment to a read-out means denoting presence, amount, or monitored level of the said one or more selected components in a liquid medium into which the support member is dipped to contact both electrodes.

small size of the throwaway electrode and of the permanent circuitry/readout components, of easy assembly and disassembly even by juvenile or elderly lay users. The relatively expensive permanent circuitry/readout components should, despite their small size, be of a form which minimizes loss or damage. The device should display readings which are visible and understandable to a non-expert user.

5 We have now found that these and other objects can be met by assembly of the circuitry/readout components into a housing resembling a pen or digital-watch.

Such an assembly of circuitry and display means for use in producing a readout value as a diagnostic aid in human or veterinary medicine, is housed in a pen-like hollow elongate housing having (a) at one end an electrically conductive socket suitable to receive the outer end of at least one detachable test member
10 capable of producing an electrical signal correlating with a physiological parameter to which the test member is selectively sensitive and (b) towards the other end a digital read-out window for exhibiting a numerical value corresponding to the parameter. A thermister may also be used for temperature compensation.

The pen-like assembly as defined above can be used in combination with an attached test member, and there can be provided a kit of interrelated parts of such an assembly with a plurality of test members
15 suitable for one-off use.

The term "pen-like" is a general limitation on size and shape. In functional terms, its characteristics are such that it can be held near the socket between the thumb and the nearer one or two opposed fingers, with the elongate body resting on and extending beyond the forefinger, but not to an extent that prejudices fine
20 control of the socket end by the thumb and fingers. In numerical terms it can be from 10 to 30 cm. long and from 0.5 to 3 cms across its maximum transverse dimension; more usually it will be from 12 to 20 cms. long and from 0.8 to 1.5 cms. across. It can be generally circular, or polygonal, in cross-section. Each detachable test member is usually a small-scale enzyme-coated sensor electrode, of the type discussed in the earlier Patent Applications listed above, and especially such an electrode where the enzyme is
25 specifically glucose-catalyzing whereby diabetic conditions can be measured. It may be a flat external strip electrode dimensioned to operate on a small, non-expressed, blood droplet. The socket arrangement will vary accordingly.

Two or more sensor electrodes may be incorporated into a single test member. Again, the socket arrangement will vary accordingly.

30 The readout means will typically be a conventional seven-segment display window towards the rearward end of the "pen" as in conventional pen/watches. In the case of the multiple sensor embodiment described in the preceding paragraph the display may be switchable between each sensor's discrete monitoring circuit, both the display and a single monitoring circuit may be switchable between sensors, or, a specific display may be provided for each of the sensors present.

35 Such equipment is of course particularly adapted for use with the non-invasive strip sensor defined above.

Another form of equipment for use with the sensor electrode systems is so-called "desk-top" equipment, i.e. for general but skilled use in a general clinic.

In this aspect, the aim is to provide suitable equipment for a practitioner of "desk-top" scale and
40 complexity and while use of such equipment may be envisaged for any enzyme-catalysable component (with suitable electrode systems) for convenience this specification will refer to glucose determination as typical.

In the operation of a glucose sensor a number of relevant technical points and advantages should be considered. It will be appreciated that these points are of general relevance and should by no means be
45 restricted in this application to particular design features: in other words, they apply generally to the embodiments listed above, and especially to those embodiments insofar as they deal with equipment and methods for glucose sensing. These features are:-

1 Constructional Features

50

(a) Membrane cover for electrode

Although the enzyme electrode should be in electrical contact with the liquid, it may be found valuable to exclude the sensor from interfering contact with larger molecules or tissue fluid components. This can be
55 done by a covering or surrounding membrane, depending on electrode geometry. Heat-shrinkable thin polymer tubing can be used as, or in connection with, such membranes.

The membranes can be polymerised in situ (e.g. cellulose acetate). A particular valuable membrane is formed by polycarbonate, especially those polycarbonates sold under the Trade Marks "NUCLEOPORE" or

In a preferred embodiment of the invention, the electron-transfer electrode is poised at a fixed potential against a reference electrode, and the current flowing in the electron-transfer electrode is measured.

The particular electron transfer electrode may be selected from a range of electrodes including those employing the following enzymes

5

	<u>Enzyme</u>	<u>Substrate</u>
	<u>Flavo-proteins</u>	
10	Pyruvate Oxidase	Pyruvate
	L-Amino Acid Oxidase	L-Amino Acids
	Aldehyde Oxidase	Aldehydes
15	Xanthine Oxidase	Xanthines
	Glucose Oxidase	Glucose
	Glycollate Oxidase	Glycollate
20	Sarcosine Oxidase	Sarcosine
	Lactate Oxidase	Lactate
25	Glutathione Reductase	NAD(P)H
	Lipoamide Dehydrogenase	NADH
30	<u>PQQ Enzymes</u>	
	Glucose Dehydrogenase	Glucose
	Methanol Dehydrogenase	Methanol and
35		other Alkanols
	Methylamine Dehydrogenase	Methylamine
40	<u>Haem-Containing Enzymes</u>	
	Lactate Dehydrogenase	Lactate
	(Yeast Cytochrome b ₂)	
45	Horse-radish Peroxidase	Hydrogen Peroxide
	Yeast Cytochrome c Peroxidase	Hydrogen Peroxide
50	<u>Metalloflavoproteins</u>	
	Carbonmonoxide Oxidoreductase	Carbon Monoxide
55	<u>Cuproproteins</u>	
	Galactose Oxidase	Galactose

Figure 4 shows a longer strip, and Figure 5 shows the inner end of a strip held between two resilient metal contact strips.

In Figure 6 a 2 cm length of electrically insulating polymer for example MYLAR or TEFLON (a polyfluorocarbon) 0.3 mm square in transverse cross-section is provided with a palladium-silver conductive electrode 31, on the front surface as shown, and a second, smaller electrode 32, on the back as shown in dotted lines. In each case conductive lines 33 and 34 respectively, were formed simultaneously with the electrodes.

On the front electrode 31 is painted a mixture of toluene, 1,1'-dimethyl ferrocene and graphite, formed by mixing a solution of the toluene and 1,1'-dimethylferrocene and a slurry of toluene and graphite. It is believed that the ferrocene is adsorbed on to graphite particles. After drying the mixture forms a layer 35. A layer 36 of glucose oxidase is then immobilised on the graphite surface by carbodiimide immobilisation, known per se (enzyme adsorption can also be used). The electrode may then be covered, on both sides, with a semipermeable membrane of cellulose acetate (or polyurethane), not shown, to block large interfering species from contact with the electrode.

The square section of the support helps in the painting of slurry, or the enzyme-attachment stages, in keeping the electrodes 31 and 32 distinct.

The small scale electrode so produced could be used per se but is especially valuable for incorporation into a standard gauge needle, giving a blood-glucose reading using the same invasive member as the eventual injection.

When producing such small scale needles, the exact sequence of steps can vary. For example, graphite could first be painted on, and a solution of the mediator (ferrocene, etc) in toluene then be applied by dipping into the graphite. Likewise, the enzyme can be applied in solution for adsorption. There is therefore a danger that the reference electrode e.g. silver/palladium could be adversely affected by the solvents or solutes used.

Figure 7 shows the key steps of a procedure which can be used to advantage in the fabrication of these microelectrodes.

The reference electrode 37 and its conductive lead-out strips 38 are formed in silver/palladium on TEFLON base 39. Similarly, an electrode support 40 and lead-out strip 41 are formed in silver/palladium on TEFLON base 42. Only this base 42 and its electrode support are then subjected to (a) painting on a graphite slurry in toluene (b) dipping in 1,1'-dimethylferrocene solution in toluene and (c) contacting with the enzyme to absorb e.g. glucose oxidase into the active layer 43. Thereafter the bases 39 and 42 are glued or held, side-by-side, their general rectangular cross-section facilitating such positive location. Back-to back location is also possible.

As before, the finished assembly may be located inside a needle bore, e.g. with extra access portions near the electrode surfaces.

Incorporation of 1,1' dimethylferrocene into the electrode

A solution of 1,1'-dimethylferrocene in toluene was mixed into a toluene-based slurry of the graphite powder. The mixture was then painted on to the base conductor 41 and allowed to dry at 43. This provided an electrode surface that was electroactive towards glucose oxidase.

These experiments showed that "thick layer" or screen-printing technology could provide a usable base strip which could easily be coated with a stable graphite surface and that moreover the electrode surface could be made electro-active towards glucose by adsorption of a ferrocene directly into the coating mixture. In addition, the reference electrode operated satisfactorily in buffered solutions.

Figures 8a and 8b show a holder which is particularly adapted to utilise electrodes as shown in Figures 3, 4, 5 but which could if necessary utilise electrodes as shown in Figures 1 and 2, and 6 and 7 at least of the various embodiments shown.

From above the holder 81 intentionally resembles a conventional pen/watch as much as possible. It has a forward end 82, possibly rotary to tighten the walls of a flattened socket cavity 83 formed within it. A central join, a clip 84 and a press-button 85 all resemble those of a conventional pen, and digital readout-window 86 is also of a type known in pen/watches.

Inside the holder as shown by dotted lines is connection circuitry 87, possibly printed in situ, battery 88 and operating circuitry 89 behind and manufactured as a unit with the display window 86. The display can be capable of operation only when button 85 is pressed so that extra illumination can be provided if necessary.

The embodiments shown in Figures 8a and 8b especially when used in conjunction with the electrodes of Figures 3 - 5 fulfill the design criteria discussed above for such portable equipment.

The reference voltage for the embodiment shown in Figure 12, is derived from the circuit elements 501-505 which include the potentiometers 503 and 502 providing a variable voltage across the sensor 101, thereby accommodating any type of electron-transfer electrode. It is envisaged that the continuously variable resistors 142a and 142b could be replaced in certain applications by stepwise resistance switching means with each position or setting being dedicated to a particular type of electrode.

Various modifications may be made in the circuitry. For example the liquid crystal display may be replaced by a plotter or a dosage control device, or a temperature stability circuit may be incorporated.

Various modifications may also be made in constructional techniques for the electrode manufacture.

The electrode may for example be manufactured by screen printing techniques e.g. in a multi-stepped procedure comprising:-

I - screen printing of Ag/AgCl reference electrode and metal tracing.

II - screen printing of the active electrode with a printing ink comprising a colloidal carbon, glucose oxidase in buffer, and an organic polymer.

III - screen printing, spraying or dip coating to provide a membrane over the assembly.

Advantages of this method are that it is amenable to high volume automation, and is of high reproducibility.

From the above it follows that a suspension in a liquid medium of carbon together with at least one of (a) an enzyme and (b) a mediator compound capable of transferring charge to the said carbon from the enzyme when the enzyme is catalytically active can be used to fabricate electrodes, provided that the said suspension is formed as a printable and conductive ink. Preferably, both the enzyme (e.g. glucose oxidase) and the mediator (e.g. ferrocene or a ferrocene derivative) are present in the ink formulation.

Copending Application EP 84303090.9 of even date herewith entitled "Assay techniques utilising specific binding agents" is concerned with the effect on the enzyme and/or mediator electrochemical availability of specific binding agents e.g. antigens/antibodies and others. It can be embodied by specialised electrodes. Such electrodes can be fabricated using the present invention. The interested reader is referred to the published specification, EP-A-125139.

Thus, the mediator may be in the form of a mediator/hapten conjugate, i.e. be linked to a ligand material so that its activity in its charge-transferring property is a measure of further or competitive binding reaction with a specific binding agent with which the eventual electrode, having the specialised ink thereon, is contacted. A specific example is the theophylline/ferrocene conjugate described in copending Application entitled "Assay systems utilizing specific binding agents", of even date herewith. Other mediator/enzyme/ligand systems can also be utilized in the specialized ink.

More generally in this respect, the area of first electrode material may comprise (a) a ligand such as an antibody (b) an antiligand, such as a hapten, with specific binding properties thereto (c) the mediator, conjugated to either (a) or (b) and electrochemically active only when (a) and (b) are not specifically bound. Such an electrode is useful in assay of a system which unbinds (a) and (b), at least in part, thereby to provide mediator for the enzyme/substrate reaction.

Claims

1. A suspension formed as a printable and conductive ink for use in the fabrication of electrodes, the suspension comprising a liquid medium containing (a) carbon, together with at least one of (b) an enzyme and (c) a mediator compound.
2. A suspension as claimed in claim 1 in which both an enzyme and a mediator are present.
3. A suspension as claimed in claim 1 or 2, in which the enzyme is glucose oxidase and the mediator is ferrocene or a ferrocene derivative.
4. The use by printing of a suspension as claimed in any preceding claim, in the fabrication of an electrode.
5. A method of manufacturing a sensor electrode system which is generally elongate and thereby manipulable, the sensor electrode system comprising an electrode carrier in the form of a flat strip carrying a working electrode and a reference electrode in spaced non-contiguous relationship, in which method an enzyme and/or a mediator are applied to the flat strip by printing a suspension as defined in any of claims 1 to 3.

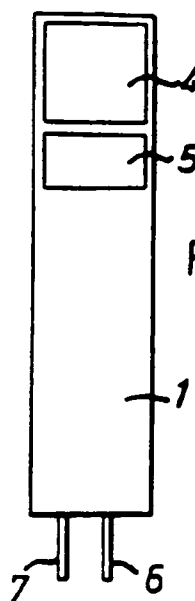


FIG.1

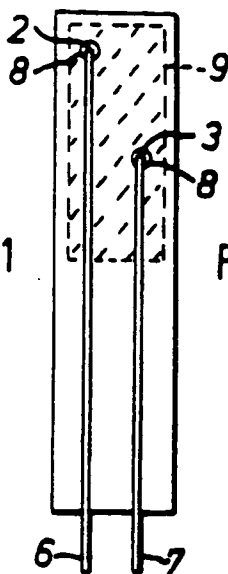


FIG.2

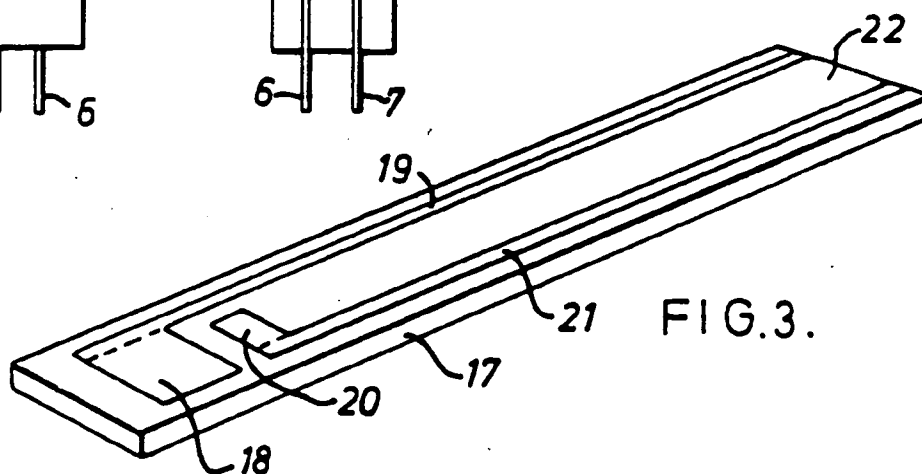


FIG.3.

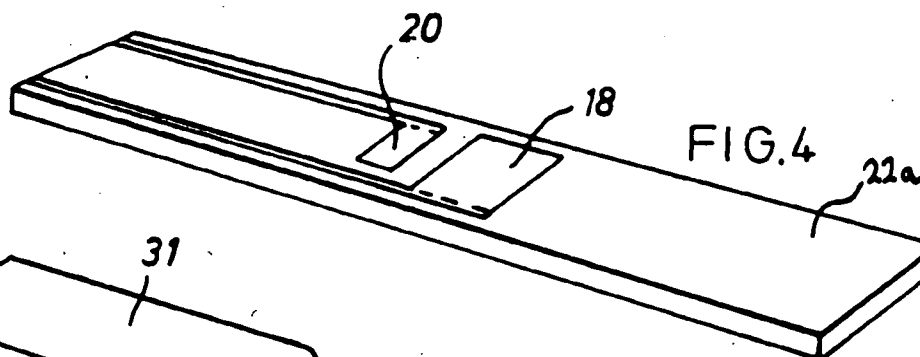


FIG.4

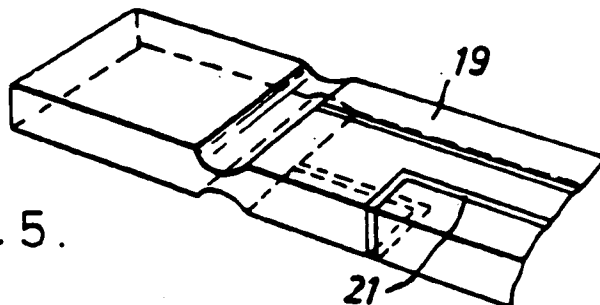
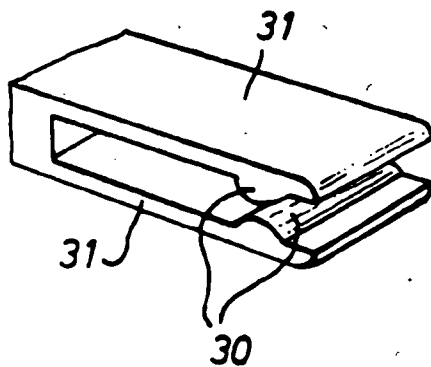
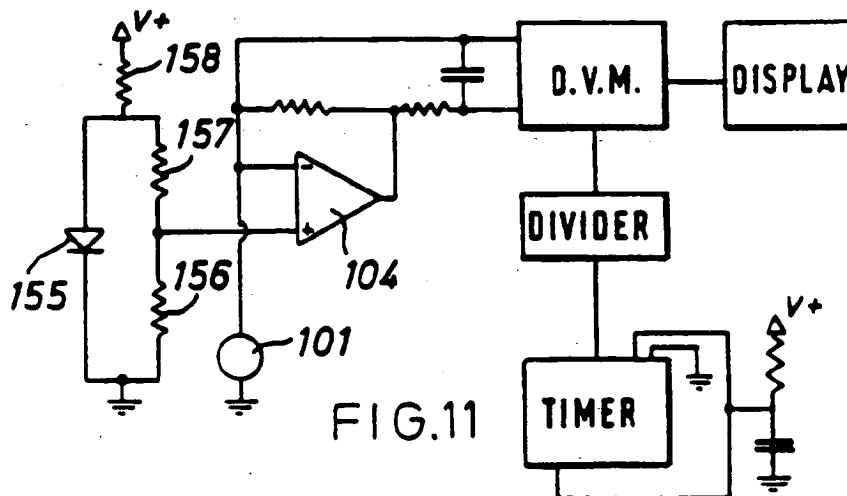
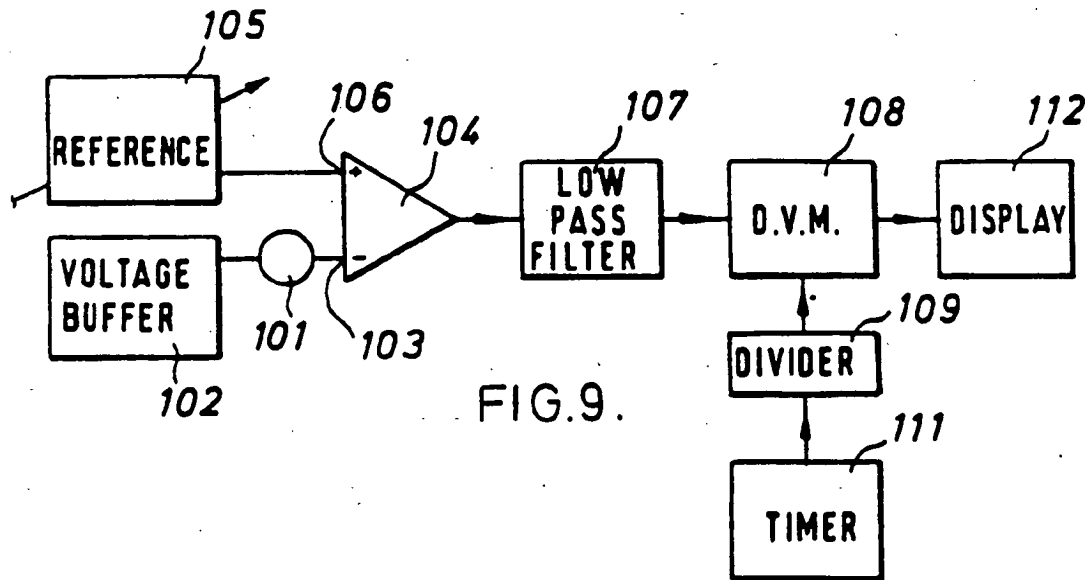
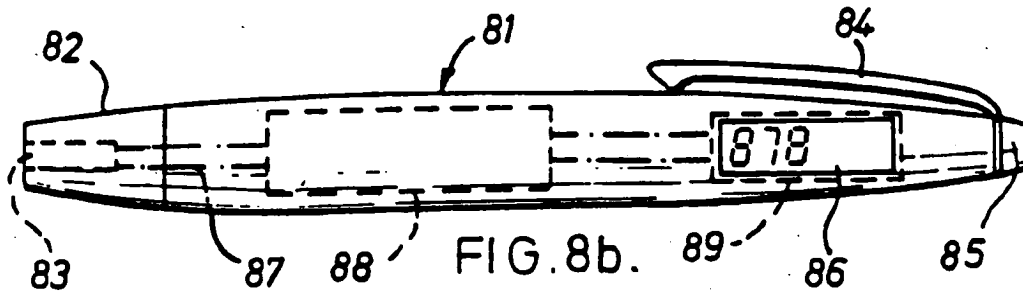
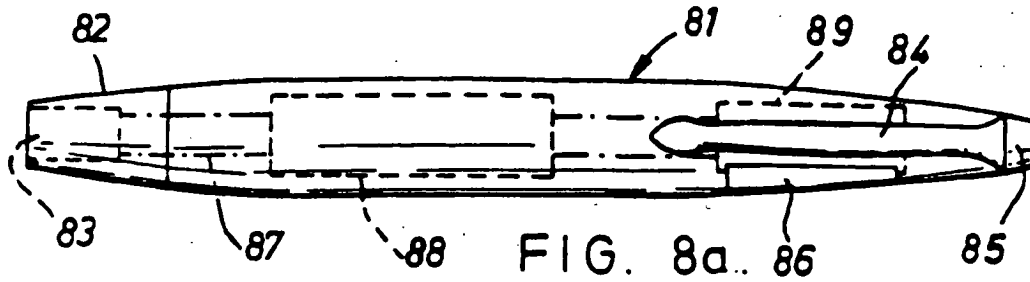


FIG.5.



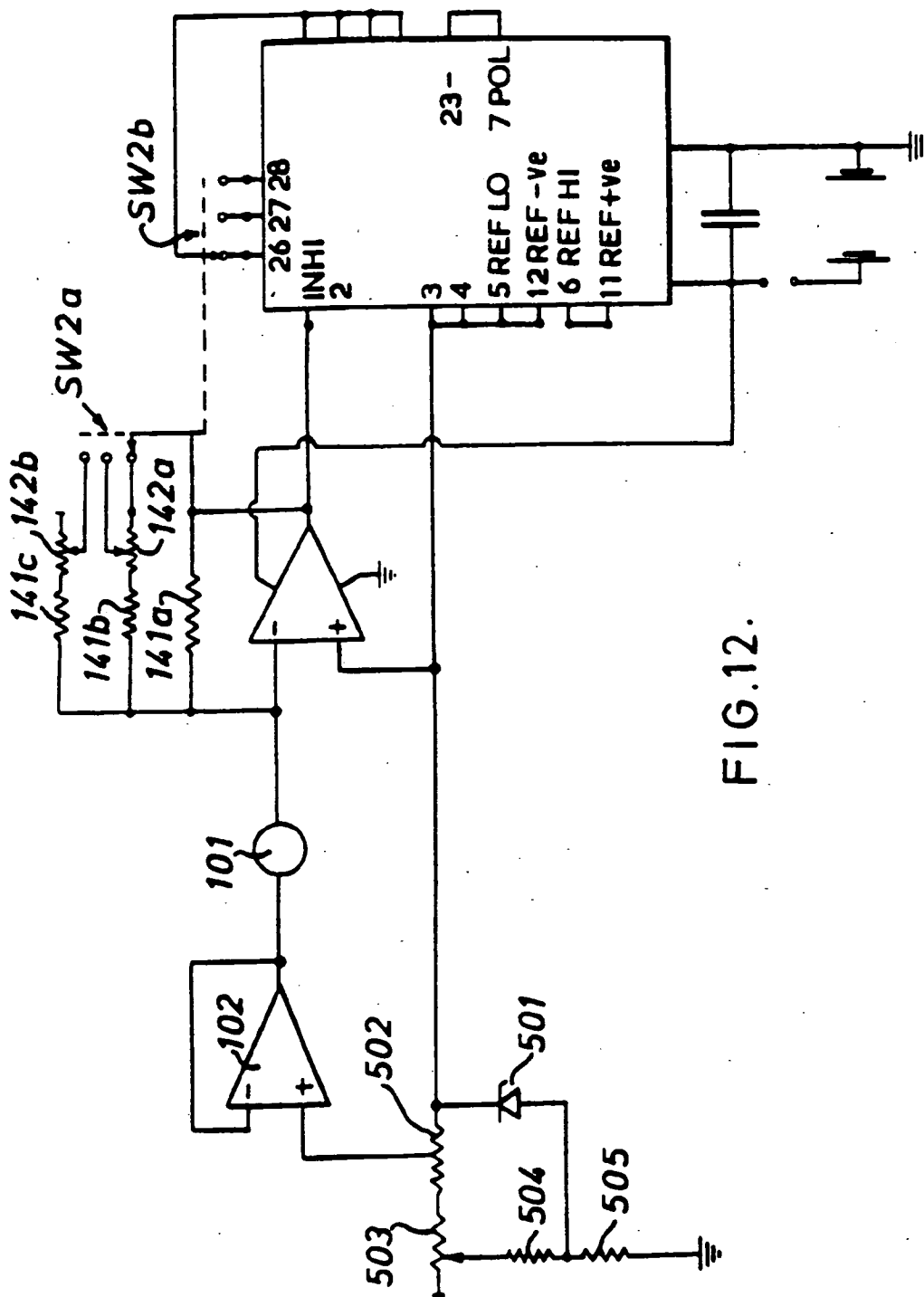


FIG.12.